



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Planar sucrose substrates for investigating interfaces found in molten chocolate

Citation for published version:

Manasi, I, Arnold, T, Cooper, JFK, Van Damme, I, Dong, C, Saerbeck, T, Stenning, GBG, Tellam, J & Titmuss, S 2019, 'Planar sucrose substrates for investigating interfaces found in molten chocolate', *Food Structure*, vol. 22. <https://doi.org/10.1016/j.foostr.2019.100128>

Digital Object Identifier (DOI):

[10.1016/j.foostr.2019.100128](https://doi.org/10.1016/j.foostr.2019.100128)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Food Structure

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



Planar Sucrose Substrates for Investigating Interfaces Found in Molten Chocolate

Iva Manasi^a, Tom Arnold^b, Joshaniel F. K. Cooper^c, Isabella Van Damme^d, Chuchuan Dong^c, Thomas Saerbeck^e, Gavin B. G. Stenning^c, James Tellam^c, Simon Titmuss^{a,*}

^a*School of Physics and Astronomy, University of Edinburgh, James Clerk Maxwell Building, Edinburgh EH9 3FD, UK*

^b*European Spallation Source ERIC, Box 126, SE-221 00 Lund, Sweden*

^c*ISIS Neutron and Muon Source, Science and Technology Facilities Council, Rutherford Appleton Laboratory, Didcot OX11 0QX, U.K.*

^d*Mars Chocolate UK Ltd., Slough SL1 4LG, UK*

^e*Institut Laue-Langevin, CS 20156, 38042 Grenoble Cedex 9, France*

Abstract

We present planar substrates suitable for investigating the sucrose/triglyceride fat interfaces found in molten chocolate with surface science techniques. The planar sucrose substrates are produced by spin coating sucrose onto hydrophilic, silicon oxide-capped, silicon substrates from millimolar aqueous solutions of sucrose. We present the characterisation of the sucrose film thicknesses and crystallinity using X-ray reflectivity and grazing incidence X-ray diffraction, respectively. These sucrose-coated substrates can be used in flow cells for Quartz Crystal Microbalance with Dissipation (QCM-D) and neutron/X-ray reflectivity measurements, through which triglyceride oils containing the surfactants commonly used in chocolate manufacture can be flowed. This provides a well-defined, planar, sucrose/triglyceride interface, which can be used to probe the solid/liquid interfaces that are found in molten chocolate at the molecular level.

Keywords: chocolate, sucrose, spin coating, triglyceride, neutron reflectivity, X-ray reflectivity

1. Introduction

Chocolate is a dense suspension ($>65\%$ w/w) of solids, mainly sucrose, milk solids and cocoa, in a continuous fat phase of cocoa butter, which is mainly composed of triglycerides,

*Corresponding author

Email addresses: I.Manasi@sms.ed.ac.uk (Iva Manasi), tom.arnold@esss.se (Tom Arnold), jos.cooper@stfc.ac.uk (Joshaniel F. K. Cooper), 1vd@protonmail.com (Isabella Van Damme), chu-chuan.dong@stfc.ac.uk (Chuchuan Dong), saerbeck@ill.fr (Thomas Saerbeck), gavin.stenning@stfc.ac.uk (Gavin B. G. Stenning), james.tellam@stfc.ac.uk (James Tellam), simon.titmuss@ed.ac.uk (Simon Titmuss)

with 95% of the fatty acid tails being derived from palmitic, stearic and oleic acids [1]. Inter-particle interactions between the solid particles affect the stability of the suspension and also control how the particles flow past each other, thereby controlling the rheology of molten chocolate. The rheology of molten chocolate plays an important role in the manufacture of different types of chocolates: a low yield stress is desirable for enrobed products, in which the chocolate forms a thin shell around a solid core, whereas a high yield stress can be favourable for moulded chocolates [2].

It has been found empirically that by using combinations of the food grade surfactants, lecithin and polyglycerol polyricinoleate (PGPR), in varying ratios, the rheological properties, as characterized by the viscosity and yield stress, of molten chocolate can be controlled [3]. It has also been established that the rheology of dense suspensions can be modified by tuning the nature of the inter-particle interactions [4], so it is hypothesized that adsorption of the surfactants used in chocolate manufacture tunes the inter-particle interactions between the solid grains, controlling the suspension rheology.

Rheology studies have shown that dense suspensions ($>50\%$ v/v) of sucrose in triglyceride oils (soybean oil, sunflower oil and commercial medium chain triglycerides) exhibit rheological properties similar to that of molten chocolate, where a yield stress is observed followed by shear thinning before reaching Newtonian flow at high shear rates [5]. On the basis of this observed rheological behaviour, such dense suspensions of sucrose in triglyceride have been widely accepted as a suitable model system for studying the effect of surfactants on the rheology of molten chocolate [6, 7].

Recently, AFM studies on grains of sucrose extracted from model chocolate suspensions (and then washed with acetone) have suggested that PGPR forms pillow-like deposits, which separate the sucrose particles by steric hindrance[8], whilst lecithin was found to be inhomogeneously distributed across the surface of the sucrose grains in layers up to 38 nm in thickness [9]. AFM studies are unable to resolve the internal structure and composition of these surfactant deposits at the molecular level. Furthermore, although the sucrose grains used in these AFM studies, have been extracted from a model chocolate suspension, the triglyceride oil has been washed away, hence the structure of the layers observed may differ from those present when the interface is solvated by the triglyceride oil phase. We suggest that detailed structural and compositional information obtained from sucrose/triglyceride interfaces is the key to fully understanding the mechanism of the dramatic lowering of the yield stress observed for mixtures of PGPR and lecithin. Understanding the structure-function basis for this mechanism is crucial in attempts to rationally design alternative surfactant combinations.

The ability of neutrons and synchrotron X-rays to penetrate condensed phases give small-angle scattering and reflectivity techniques, which exploit these radiations, unique benefits when trying to probe the structure and composition of such buried interfaces. Some information about the adsorption of surfactants from the oil phase of these dense suspensions onto the surfaces of the sucrose particles can be obtained by using small angle scattering of X-rays and neutrons directly from the dense suspensions. Whilst this is an approach that we have exploited [10], laboratory-based approaches to probe the adsorption of surfactants at solid/liquid interfaces, such as QCM-D and sessile drop tensiometry, cannot be

performed directly on the surfaces of sucrose grains as they require planar substrates with areas $\gtrsim \text{cm}^2$. Furthermore, more detailed structural information at the solid/liquid interface can be provided by neutron and X-ray reflectivity techniques than by small angle scattering from dense suspensions. The detailed experimental structural information derived from planar sucrose interfaces is complementary to that obtained from the multi-scale simulations used by Greiner and co-workers [11, 12].

The approach we describe in this manuscript has facilitated the execution of a self-consistent strategy, in which neutron and X-ray reflectivity studies are used to provide the detailed molecular compositional and structural information of the adsorbed layers formed from PGPR/lecithin surfactant mixtures at sucrose triglyceride interfaces. The application of neutron and X-ray reflectivity to investigate the adsorption of surfactants at solid/liquid interfaces is well-established [13] but typically uses substrates such as silicon/silicon oxide. As the suspensions studied by Arnold [6], in which glass beads replaced grains of sucrose, showed different rheology to model chocolate suspensions, it was necessary to produce and characterize a planar substrate bearing a sucrose interface before the power of these reflectivity techniques could be applied to study the adsorption of surfactants relevant to understanding the rheology of molten chocolate. The grazing angle of incidence used in these reflectivity techniques necessitates the use of planar substrates that are typically several cm^2 in area, in addition to being flat and smooth at the nm level [14].

We have developed an experimental approach, using spin coating, to produce planar thin films of sucrose on silicon oxide-capped silicon substrates that can be used to carry out adsorption studies using QCM-D, contact angle tensiometry and neutron/synchrotron X-ray reflectivity. We describe this approach and show the characterisation of the sucrose film thickness, roughness and crystallinity. We also give examples of the neutron and X-ray reflectivity that can be measured from the interface between this sucrose film and triglyceride oils that can serve as a model for the cocoa butter phase in molten chocolate. The adsorption behaviour of the surfactants used in chocolate manufacture measured using these planar sucrose substrates and the implications for a structural basis for the control of the rheology of molten chocolate will be reported in future publications.

Although chocolate provides the context for our investigations, a recent review addressing the role of non-fat ingredients on confectionary fat crystallization in general, identified a lack of information on the structure-function relationships for emulsifiers and their interactions with fats [15], so we think that our approach will be of interest to the wider food science community. Trying to identify common phenomenology across a range of food systems is the basis of physics-based approach to food science. As such a physics-based approach seeks to identify generic behaviour from minimal models, our studies use two separate single-component triglyceride oil phases (with purity $>99\%$) as models for molten cocoa butter: the long-chain unsaturated triolein (TO) and the medium chain saturated glyceryl trioctanoate (GTO). The rheology of sucrose/triglyceride oil suspensions formulated using these two different oils is qualitatively similar [10], suggesting that the interfacial films present at the sucrose/oil interfaces modulate the particle-particle interactions in a similar manner. As GTO is more easily deuterated than TO, more extensive investigations using neutron reflectivity are feasible by using both GTO and TO.

2. Materials and Methods

The silicon substrates used for spin coating were either 55 mm diameter disks (thickness 5 mm) or 1" diameter wafers sourced from PI-KEM Ltd. In the case of spin coating onto QCM-D sensors, silicon oxide Q-Sense E4 sensors were used. The sucrose used for spin coating was crystalline icing sugar, sourced from British Sugar with an average particle size $\sim 10\ \mu\text{m}$, dissolved in Millipore Milli-Q water, with resistivity $18.2\ \text{M}\Omega\ \text{cm}$ and total organic content = 4 ppb. Pure ($\geq 99\%$) glyceryl trioctanoate (GTO) and glyceryl trioleate (TO), purchased from Sigma Aldrich UK, were used for solid/liquid contact angle studies, QCM-D and neutron reflectivity (NR). The deuterated glyceryl trioctanoate (d-GTO) used for NR was kindly provided by the ISIS Deuteration Facility at Rutherford Appleton Laboratories, Oxford, UK.

Spin coating is a commonly applied technique to deposit uniform thin films, ranging from a few nanometres to a few microns in thickness, onto flat substrates [16]. The silicon substrates were cleaned first using piranha solution, a 5:4:1 (by volume) mixture of water, concentrated sulphuric acid and 30% hydrogen peroxide, followed by either uv ozone treatment for 30–50 mins or oxygen plasma for 1 min. The uv ozone or oxygen plasma treatment is necessary to create a hydrophilic, high surface energy surface onto which the sucrose is spin coated, in order that the spin coating solution uniformly wets the substrate. For spin coating onto QCM-D sensors, the Q-sense E4 sensors were cleaned first with 2% SDS solution followed by uv ozone. Sucrose solutions of concentrations in the range 30 – 1800 mM were made by dissolving sucrose in Milli-Q water and sonicating for 10 minutes. This solution was then spin coated onto the cleaned silicon substrates at 4000 rpm for 1 minute and dried under vacuum at 70°C overnight.

To characterise the thickness and roughness of the spin coated sucrose films, specular X-ray reflectivity (XRR) was measured on the Rigaku Smartlab Diffractometer in the Materials Characterisation Laboratory at ISIS Neutron & Muon source using X-rays of wavelength $1.54\ \text{\AA}$ (Cu-K- α) incident onto the air/sucrose interface from air. Using 2θ in the range $0.1 - 5^\circ$, a Q -range of $0.01 - 0.3\ \text{\AA}^{-1}$ was obtained. The crystallinity of the films was investigated by Grazing Incidence X-ray Diffraction (GIXRD), using the same instrument, with 2θ in the range $5 - 60^\circ$ and an angle of incidence $\Omega = 0.7^\circ$. The QCM-D measurements were made using a Biolin Scientific QCM-D in the Biology Laboratories at ISIS Neutron & Muon source. Sessile drop interfacial tensiometry experiments were conducted using the Drop Shape Analyser (DSA) from KRÜSS GmbH. NR from the sucrose/triglyceride interface was measured using D17 at the ILL, Grenoble, France [17] with a horizontal scattering geometry (vertical surface) and OFFSPEC at ISIS Neutron & Muon source, Oxford, UK [18] with vertical scattering geometry (horizontal surface). XRR was measured using I07 at Diamond Light Source, Oxford, UK [19] with vertical scattering geometry (horizontal surface). The NR data presented here was measured on D17 using the divergent beam method [20, 21], which enabled measurements to be made from comparatively small ($28 \times 28\ \text{mm}$) samples, without drastically extending the measurement time. Neutron wavelengths in the range $2\text{--}27\ \text{\AA}$ and incidence angles of 0.8° and 2.4° allowed the reflectivity to be measured for $0.006 \leq Q \leq 0.26\ \text{\AA}^{-1}$. On I07 using $24.4\ \text{keV}$ X-rays with wavelength of $0.51\ \text{\AA}$, data was

collected in the Q -range spanning $0.017 \leq Q \leq 0.32 \text{ \AA}^{-1}$ by changing the incident angle and using different levels of attenuation for the incident beam. The XRR and NR data has been analysed using simple layer models, in which each layer is characterised by a thickness, scattering length density (SLD), and a Nevot-Croce (Gaussian) roughness in Motofit [22] and RasCAL [23]. Confidence bands on the fit parameters were determined using RasCAL's inbuilt Bootstrap analysis function.

3. Results and Discussion

3.1. Sample Preparation

Figure 1(a), shows photographs of spin coated sucrose films of varying thicknesses between 10 nm and 550 nm. SEM and AFM images, shown in Figures 1(b) and 1(c) respectively, show that the films are globally flat on a sub-micrometer scale. The rms roughness obtained for a $100 \times 100 \mu\text{m}^2$ sample area was 1.9 nm^1 .

Under ambient lab atmosphere, these sucrose films degrade in 4-6 hours due to absorption of moisture, but they can be stored for days (upto 2 weeks has been tested) under vacuum. When sealed into our flow cells, containing triglyceride oils, they were stable for at least 2 days, which allowed the neutron and synchrotron X-ray reflectivity measurements to be completed without degradation of the sucrose layers. All samples were prepared directly before the measurements, stored under vacuum at 70°C prior to sealing into flow cells, which were then filled with triglyceride oil.

3.2. Characterisation of Sucrose Thin Films

The thickness of the spin coated sucrose films with thicknesses less than 300 nm was measured using XRR [25]. For films thicker than 300 nm the Kiessig fringes become too closely spaced to be resolved, and so the thickness of these thicker films has been determined by dividing the mass per unit area obtained by applying the Sauerbrey [26] equation to the frequency shift observed in QCM-D measurements on sucrose-coated sensors in air, by the density of the film [27]. As the d -spacing obtained from GIXRD suggests that the unit cell volume, and hence the film density, will be within 10% of the bulk values for sucrose (density= 1.59 g/cm^3), this value has been used for the film density.

Specular XRR profiles measured from sucrose thin films spin coated onto silicon oxide-capped silicon substrates, with the X-rays incident from air onto the sucrose, are shown in Figure 2(a). The circles show the measured data points and the solid lines show the fit to the data using the SLD profile given in Figure 2(b). The silicon substrates are capped by a thin oxide layer as a result of the uv ozone/plasma treatment, as is illustrated in the inset layer profile of Figure 2(b) with the X-ray scattering length densities (SLD) of the various components given in Table 1. Fitting the data to the model SLD profile results in sucrose

¹The AFM texture profile can be decomposed into waviness and local roughness components. The waviness is typically on the $\sim 10 \mu\text{m}$ scale and is a consequence of the surface topography convoluted with the finite size of the stylus tip [24]. The rms roughness quoted here was evaluated after removal of waviness from the measured profile, as is typical when evaluating roughness from AFM profiles.

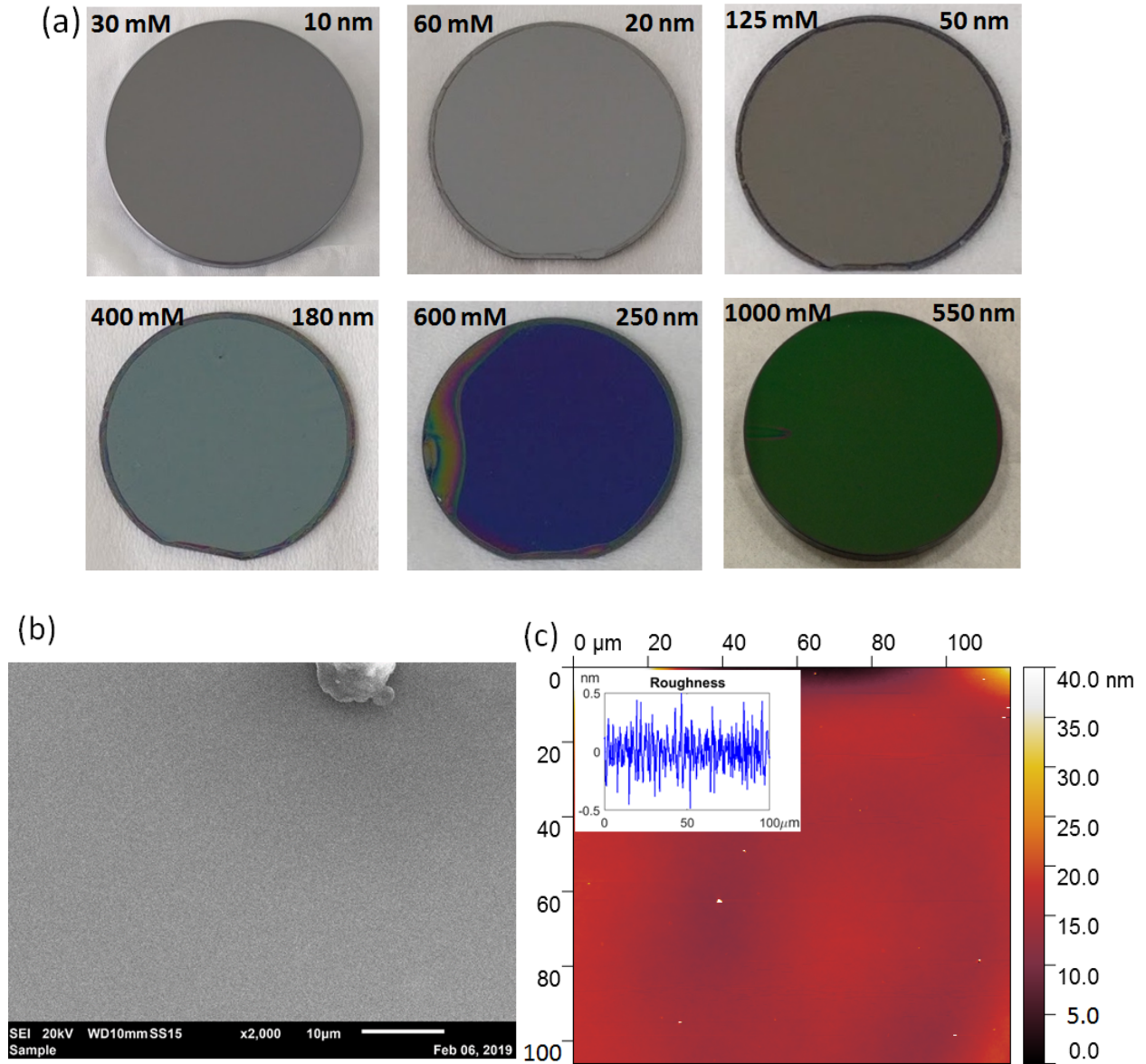


Figure 1: (a) Photographs showing sucrose films spin coated onto silicon substrates from aqueous sucrose solutions with concentrations ranging from 30 mM to 1000 mM producing films with thicknesses ranging from 10 nm to 550 nm. (b) SEM image of 50 nm thick sucrose film. (c) AFM image of $\sim 1 \mu\text{m}$ thick sucrose film with the inset showing the roughness along a typical line profile.

layer thicknesses in the range 10 – 300 nm depending on the concentration of the spin coating solution, as shown by the red square data points on Figure 2(c) and summarised in Table 2.

The thicknesses determined from QCM-D measurements made on sucrose films spin coated onto sensors using solutions ranging from 125 mM to 1800 mM are summarised in

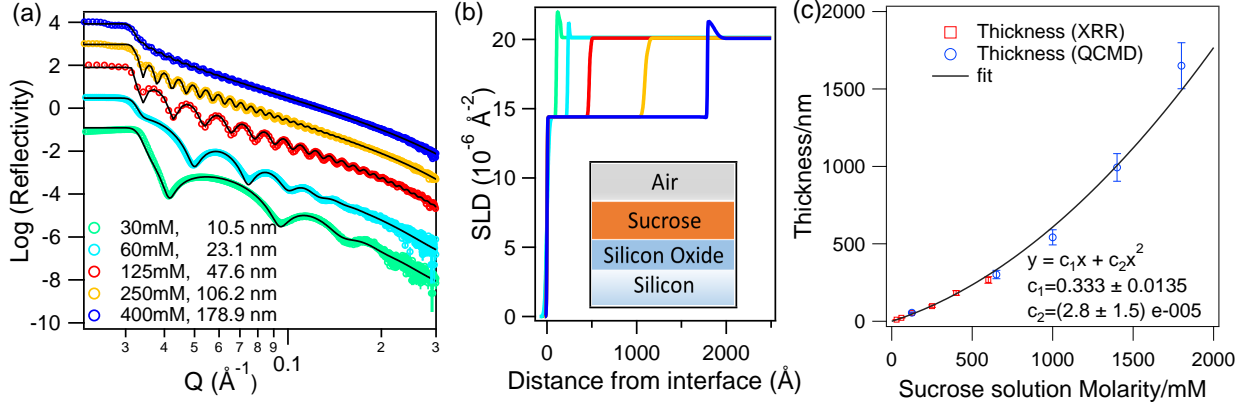


Figure 2: (a) XRR from sucrose films, spin coated onto silicon oxide-capped silicon substrates, with thicknesses from 10 to 180 nm, with the hollow circles representing the measured XRR profile and the black solid line showing the fit using a simple layer model. (b) SLD profiles for the model fits with the inset showing the arrangement of the layers. (c) A plot showing the dependence of spin coated sucrose film thickness on the concentration of sucrose in the spin coating solution.

Table 2 and shown by blue circular data points on Figure 2(c). The thickness determined using XRR for films deposited using 125 mM sucrose solution is an average over 28 samples, with a standard deviation of 4.2 nm, whereas the thicknesses determined for the other films are an average over 2 or 3 samples. Overall the sample-to-sample variability is 8%, which we attribute to small differences in the volume of solution that is spread before spinning and in the wetting properties of the silicon oxide layer caused by variations in the uv ozone or plasma treatment used to create the hydrophilic surface.

Both the XRR and QCM-D results show that the spin coated film thickness increases as the concentration of the sucrose solution used for spin coating increases. The combined data are best described by a second-order polynomial, in which the quadratic dependence is attributed to the effect of the increase in the viscosity of the solution with increasing concentration, which means that the amount of solution that is spun off (at constant spinning speed) decreases with increasing solution concentration. Film thickness can also be controlled by varying the spinning speed, ω , with thickness varying as $1/\sqrt{\omega}$ [28]. The roughness of the sucrose films determined by fitting the specular XRR is in the range 0.5 – 1.0 nm, depending on film thickness.

Our subsequent studies of the sucrose/triglyceride interface using XRR and NR used sucrose films with thicknesses ~ 50 nm, for which the roughness of 0.9 nm is sufficiently low to be able to measure specular reflectivity profiles. Although the thicker films have a lower roughness at the air/sucrose interface, reflectivity profiles are more sensitive to any changes in the interfacial layer, when the interfacial layer and sucrose layer thicknesses are comparable. The 50 nm thick sucrose films provides a compromise between this sensitivity and an acceptable interfacial roughness.

The physical properties of sucrose depends on whether it is crystalline or amorphous [29],

Table 1: X-ray and neutron scattering length density for various chemicals.

Chemical	X-ray SLD $\times 10^{-06} \text{ \AA}^{-2}$	neutron SLD $\times 10^{-06} \text{ \AA}^{-2}$
Silicon	20.1	2.07
Silicon oxide	22.5	3.47
Air	0	0
Sucrose	14.4	1.72
h-GTO	9.0	0.33
h-TO	8.8	0.15
d-GTO	NA	6.6
d50-GTO	NA	3.45 ^a

(a) d50-GTO is a 50:50 mixture of h-GTO and d-GTO.

Table 2: Average thickness of spin coated sucrose films calculated from XRR (left) and QCM-D (right)

Layer thickness roughness				Layer thickness	
Conc	SiOx	sucrose	sucrose	Conc	sucrose
mM	nm	nm	nm	mM	nm
30	1.7	10.4	0.7	125	55
60	1.0	22.1	0.9	650	302
125	1.8	53.1	0.6	1000	542
250	1.1	97.8	0.5	1400	993
400	1.6	183	0.7	1800	1650
600	3.8	267	0.5		

and in chocolate, crystalline sucrose, with a low water content is used. To investigate the crystallinity of the thin films prepared by spin coating, Grazing Incidence X-ray Diffraction (GIXRD) was measured. By using a fixed grazing angle of incidence, $\Omega = 0.7^\circ$, set to be slightly larger than the critical angle for total reflection the contribution from the film relative to that from the substrate can be maximised as the penetration depth of the incident X-rays is limited.

Figure 3(a) shows GIXRD from films with thicknesses in the range 10 – 500 nm. Broad Bragg reflections are observed from the films at $q \sim 1 \text{ \AA}^{-1}$ and $q \sim 1.5 \text{ \AA}^{-1}$. For films with thicknesses below 100 nm, the reflections are difficult to resolve from the background signal from the silicon substrate. To enhance the features from the thin films, we divide the total signal by that measured from the bare silicon substrate, and this relative GIXRD intensity is shown in Figure 3(b). From this relative GIXRD intensity, it can be seen that the broad

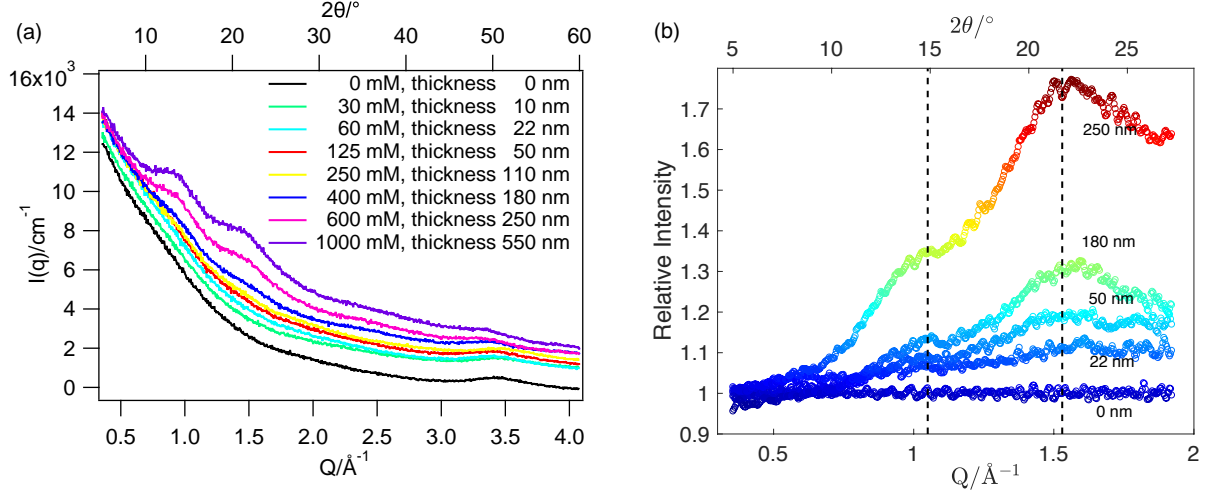


Figure 3: (a) GIXRD from sucrose films of thicknesses ranging from 10 nm to 500 nm. (b) Relative GIXRD intensity for various films with thickness < 250 nm.

peaks are still present even in the 50 nm thick film prepared from a 125 mM sucrose solution.

Crystalline sucrose is monoclinic [30] and a powder diffraction pattern measured from the sucrose used in this study suggests lattice parameters of $a = 10.48 \text{ \AA}$, $b = 8.54 \text{ \AA}$, $c = 7.58 \text{ \AA}$, $\beta = 105^\circ$, which is consistent with literature values [30]. For these lattice parameters, the broad peak observed in GIXRD at $q \sim 1.5 \text{ \AA}^{-1}$ could contain contributions from (020) and (120) reflections and that at $q \sim 1 \text{ \AA}^{-1}$ contributions from (110), (011), (101) reflections. The broad and overlapping nature of these reflections, suggests that the thin film has a polycrystalline character. That these planar interfaces have a polycrystalline character makes them comparable to the interfaces imaged with AFM from single sucrose grains extracted from chocolate-like dispersions [8, 9]. These AFM images show the surfaces feature steps, 1-2 nm in height, which the authors point out are consistent with the sucrose crystal lattice [8, 9]. Interestingly the surface planes we identify as possibly contributing to the broad GIXRD peaks observed all correspond to faces observed in the growth habit of sucrose [31]. Furthermore the *rms* roughnesses we obtained by fitting the specular X-ray reflectivity from the bare sucrose thin films in air are consistent with our planar surfaces featuring similar 1 nm height steps to those observed in the AFM studies on single sucrose grains [8, 9]. We note that a recent study that focussed on sucrose films of thickness ≤ 33 nm, concluded that their films showed no crystalline character [32]; this is in contrast to our observation of broad GIXRD peaks for the 22 nm film. This emphasizes that the details of the interfacial preparation procedure influences the crystalline character of the sucrose thin films.

3.3. Applications

The silicon blocks coated with polycrystalline sucrose films can be used for interfacial studies. Sessile drop contact angle measurements (as in Figure 4(a)) indicate TO/sucrose

and GTO/sucrose contact angles of 17.9° and 19.1° , respectively; as there is no systematic deviation of the contact angle with time, we have no evidence for adsorption of partial glycerides or free fatty acids from these $>99\%$ purity triglycerides. As the pure triglyceride oils partially wet the sucrose, with a low contact angle, it is clear that lecithin and PGPR in chocolate are not simply acting to promote wetting of the sucrose by the oil. Some information about the interfacial behaviour of the oils and surfactants present in chocolate can be obtained by measuring QCM-D from sucrose-coated sensors [10] but to fully understand why the triglyceride oils wet the hydrophilic sucrose interfaces and to understand the mechanism by which lecithin and PGPR alter the interfacial properties of sucrose, requires the molecular scale structural and compositional information to be measured at the sucrose/triglyceride interface using neutron and X-ray reflectivity [33].

To enable structural investigations of the sucrose/triglyceride interfaces using synchrotron XRR and NR, flow cells that have a similar geometry to a QCM-D flow cell have been constructed from PEEK. Figure 4(b) shows the NR cell: triglyceride oil is pumped into the square cavity at the centre of the image through the seven inlet holes on the left-hand side of the cavity and the oil leaves by the seven outlet holes on the right-hand side of the cavity; the cavity is sealed with the sucrose coated face of the silicon disc facing the triglyceride filled cavity, by means of the viton o-ring and an aluminium clamping frame; in the image the silicon block has been replaced by a quartz optical flat to enable the flow of the triglyceride oil through the cell to be visualised. By pumping at 1 mL/min we have observed that the flow is laminar, which enables the contents of the cell to be exchanged by pumping in just 1 mL of a new solution. In a NR experiment the neutrons enter through the side of the silicon disc, making a grazing angle of incidence with the sucrose/triglyceride interface from the silicon superphase.

Deuterated triglyceride oils are expensive, particularly if they are unsaturated, as in the case of TO. The low volume (~ 1 mL) cell described above, has enabled us to conduct systematic NR investigations of structure of the sucrose/oil interface from hydrogenous GTO and a 1:1 mixture of hydrogenous and perdeuterated GTO, which we label d50-GTO, as well as from hydrogenous h-TO.

Example NR profiles measured from the sucrose/d50-GTO (blue data points), sucrose/h-GTO (green data points) and sucrose/h-TO (red data points) interfaces are shown in Figure 5. Fitting the data to a simple layer model, using the neutron SLDs of various components as given in Table 1, results in sucrose layer thicknesses of 50.7 ± 0.3 nm ($\chi^2=1.2$), 60.4 ± 0.5 nm ($\chi^2 = 3.9$) and 49.5 ± 0.6 nm ($\chi^2 = 3.7$), for d50-GTO, h-GTO and h-TO interfaces respectively. This is consistent with the thicknesses measured by XRR in air for sucrose spin coated from 125 mM solution, which is the concentration of the solution used for creating these substrates. The interfacial widths on the sucrose/triglyceride interfaces are 0.7 ± 0.3 nm, 1 ± 0.5 nm and 1 ± 0.9 nm, respectively. This simple layer model does not provide a complete description of the low- Q reflectivity measured from the sucrose interfaces with h-TO and h-GTO, as is evident from the higher χ^2 values obtained from these samples.

To measure reflectivity from d50-TO, we needed to construct an even lower volume cell. We created this extremely low volume (~ 200 μ L) cell by sandwiching a 0.1 – 0.2 mm thick film of the d50-TO between two silicon discs that were separated by a kapton gasket, held in

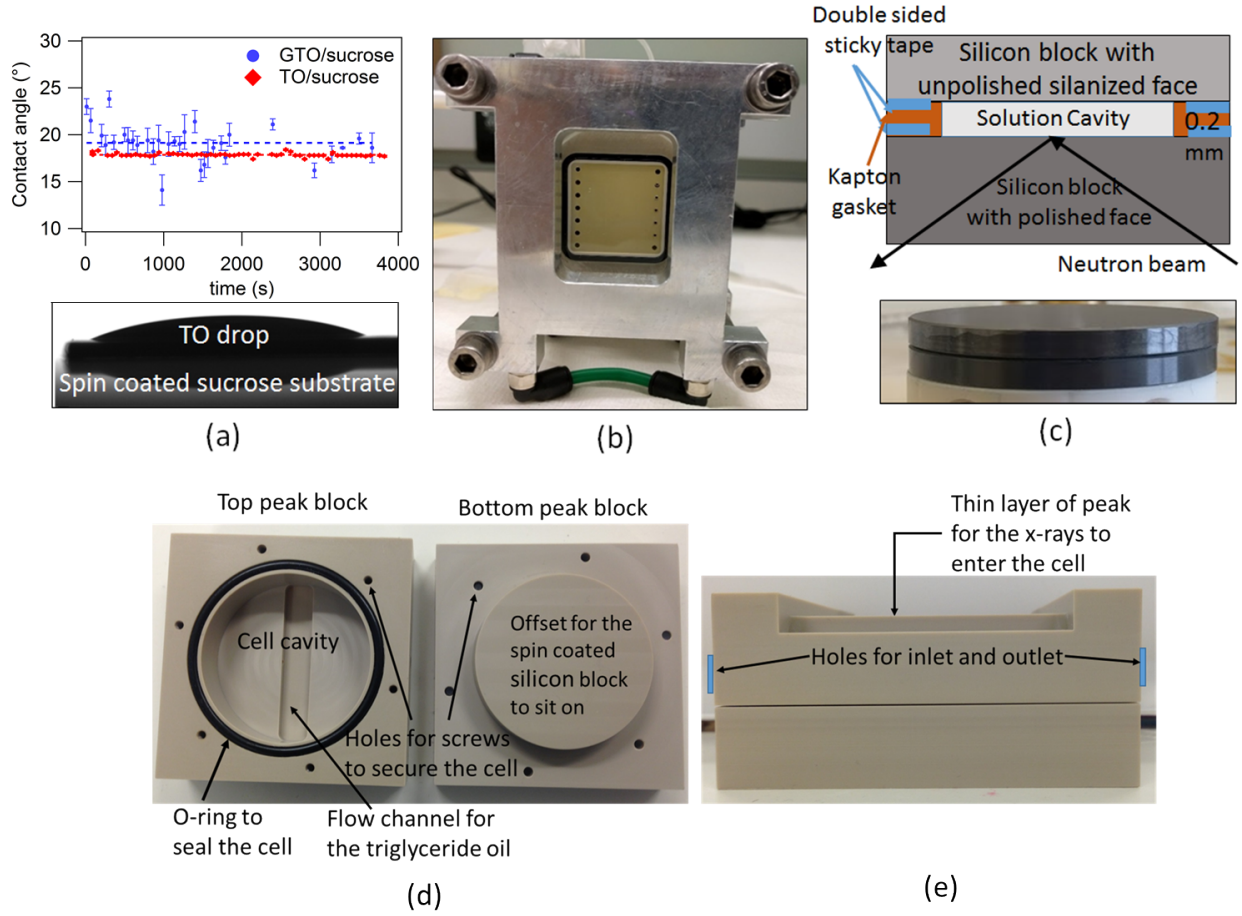


Figure 4: (a) Interfacial tensiometry showing a droplet of Glyceryl Trioleate (TO) on the spin coated sucrose substrate with a graph showing the measured contact angle for TO (red) and GTO (blue) with time. The dashed line represents the average contact angle over 1 hour; the TO measurements were taken after an upgrade to the instrument, making the error bars smaller than the data points. (b) Low volume (~ 1 mL) laminar flow cell for NR. The silicon substrate spin coated with the sucrose sits in place of the optical flat currently shown in the picture to seal the flow cell (c) Extremely low volume (~ 200 μL) sandwich cell used for NR. (d) & (e) Pictures of flow cells used for XRR.

place by double-sided sticky tape, creating an oil-filled cavity of dimensions $35\text{ mm} \times 35\text{ mm}$. One face of the cavity is provided by a 50 nm thick sucrose film, that has been spin coated onto the polished surface of a silicon disc, as in the laminar flow NR cell described above, and the other face of the cavity is provided by an unpolished silicon disc that was hydrophobised using octadecyltrichlorosilane, to passivate it. Neutrons are again incident through the side of the silicon disc with the polished face; using an unpolished face to seal the cell means that there will be no contribution to the reflectivity from that surface.

In the synchrotron XRR cell shown in Figure 4(d) & (e), the sucrose coated disc sits

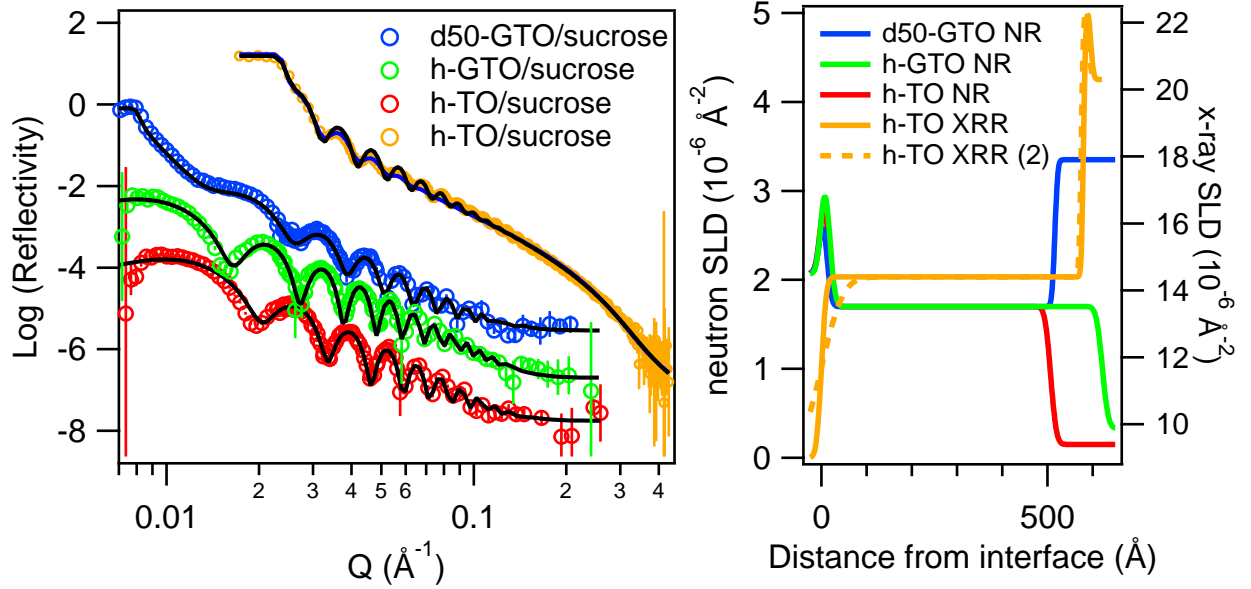


Figure 5: NR and XRR measured on D17 and I07, respectively, from the sucrose/triglyceride interface. The blue data set was NR measured from the sucrose/d50-GTO interface, the green data set NR from the sucrose/h-GTO interface, the red data set NR from the sucrose/h-TO interface and the orange data set XRR from the sucrose/h-TO interface. Left panel: Measured reflectivity profile (hollow circles) and the fit (solid lines) using a simple layer model. Right panel: SLD profile corresponding to the model fit.

face up in a two piece PEEK pocket, which is sealed by a viton o-ring. Oil flows across the sucrose surface, and the high energy X-rays are incident through the thin upper layer of PEEK and the thin layer of triglyceride oil, sandwiched between this PEEK piece and the sucrose coated silicon disc. By using these two types of cell, XRR and NR can be measured from samples that have been prepared in exactly the same way, on the same silicon discs. By using X-rays with an energy of 24.4 keV, absorption is kept sufficiently low for reflectivity profiles to be measured from the sucrose interface.

An example XRR profile measured from the sucrose/TO interface (orange data points) is shown in Figure 5. Fitting the data to a simple layer model, using the X-ray SLDs of the various components results in a sucrose layer thickness of 57.9 ± 0.8 nm resulting in a $\chi^2 = 4.5$ (blue line in left panel, dashed orange line in right-panel). The corresponding interfacial width of 4.3 ± 0.9 nm is significantly higher than that determined by fitting the NR data or the roughness of the bare sucrose surface measured by XRR in air. Using a more realistic prior parameter distribution that restricts the maximum interfacial width to 20 Å resulted in a fitted interfacial width of 8 Å but increased χ^2 to 6.2 (black line in left panel, solid orange line in right panel). The increase in χ^2 was caused by the calculated Kiessig fringes becoming out-of-phase with those measured for $Q > 0.1$ Å⁻¹ and is a clear indication that the simple layer model, using the bulk density of the triglyceride, does not provide a

complete description of the structure of the sucrose/triglyceride interfaces from which these reflectivity profiles have been measured. The complete structural characterisation of the sucrose/triglyceride interface will be the subject of a future publication [33].

4. Conclusions

We have described a method to produce low roughness sucrose thin films, with thicknesses in the range from 10 nm to $\sim \mu\text{m}$, that is based on spin coating sucrose solutions onto silicon oxide-capped silicon substrates. X-ray reflectivity measurements made at the sucrose/air interface have shown the roughness of the sucrose film of thickness 50 nm to be 1 nm, which is small enough to facilitate the measurement of specular neutron and synchrotron X-ray reflectivity from the interface between the sucrose and a triglyceride oil phase. Grazing incidence X-ray diffraction has been used to establish that the films have a polycrystalline character, which makes them a good planar model for the interfaces found between sucrose grains and the liquid component of cocoa butter found in molten chocolate.

Sessile drop contact angle measurements of triglyceride oils chosen to be suitable single component models for molten cocoa butter indicate that the oils partially wet sucrose, with a low contact angle. Preliminary fits to the neutron and synchrotron X-ray reflectivity measured from the interfaces between these sucrose thin films and the triglyceride oils are sufficiently good to determine sucrose film thicknesses that are consistent with those measured for the bare sucrose layers in air. However the deviations between the measured reflectivity and that calculated using a simple layer models, at low- Q for the NR measurements made with h-TO and h-GTO and at high- Q for the XRR measurements made with h-TO, indicate that such a simple model does not provide a full description of the interfacial structure.

The detailed molecular scale structural and compositional information that can be determined from such reflectivity techniques will be complementary to information on adsorption that can be obtained using such planar substrates in lab-based measurements such as QCM-D [10, 33] and to that provided by multi-scale simulations based on molecular dynamics [11, 12].

The methodology we have described will facilitate the generation of structure-function relations for chocolate and other food and pharmaceutical formulations that involve sucrose interfaces.

5. Acknowledgements

We thank Mars Chocolate for materials and funding to IM through a EPSRC CASE Conversion (EPSRC Grant EP/M506515/1). We also thank the Institut-Laue Langevin, ISIS Neutron & Muon Source and Diamond Light Source for the beam time (Experiment No. 9-13-642, RB1600043 and SI15202-1, respectively) and for the use of the ISIS Materials Characterisation Laboratory, Deuteration Facility and Biology Laboratory.

References

- [1] G. Talbot, Chocolate and cocoa butter - structure and composition, in: N. Garti, N. R. Widlak (Eds.), *Cocoa Butter and Related Compounds*, AOCS Press, 2012, Ch. 1, pp. 1–33.
- [2] S. T. Beckett, *The Science of Chocolate*, The Royal Society of Chemistry, 2019.
- [3] B. Schantz, H. Rohm, Influence of lecithin-PGPR blends on the rheological properties of chocolate, *LWT Food Science and Technology* 38 (2004) 41–45.
- [4] E. Irani, P. Chaudhuri, C. Heussinger, Impact of attractive interactions on the rheology of dense athermal particles, *Phys. Rev. Lett.* 112 (2014) 188303.
- [5] M. A. Rao, *Flow and Functional Models for Rheological Properties of Fluid Foods*, Springer US, Boston, MA, 2014, pp. 27–61.
- [6] G. Arnold, S. Schuldt, Y. Schneider, J. Friedrichs, F. Babick, C. Werner, H. Rohm, The impact of lecithin on rheology, sedimentation and particle interactions in oil-based dispersions, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 418 (2013) 147 – 156.
- [7] F. C. Vernier, Influence of Emulsifiers on the rheology of chocolate and suspensions of cocoa or sugar particles in oil, Ph.D. thesis, University of Reading (1997).
- [8] D. Middendorf, A. Juadpur, U. Bindrich, P. Mischnick, Afm approach to study the function of pgpr’s emulsifying properties in cocoa butter based suspensions, *Food Structure* 4 (2015) 16 – 26, food Structure and Functionality Forum Symposium 2014.
- [9] D. Middendorf, U. Bindrich, P. Mischnick, A. Juadpur, K. Franke, V. Heinz, Atomic force microscopy study on the effect of different lecithins in cocoa-butter based suspensions, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 499 (2016) 60 – 68.
- [10] I. Manasi, Structural Basis for the Rheology of Molten Chocolate: A Multi-Technique Approach, Ph.D. thesis, School of Physics and Astronomy, University of Edinburgh (2019).
- [11] M. Greiner, B. Sonleitner, M. Mailänder, H. Briesen, Modeling complex and multi-component food systems in molecular dynamics simulations on the example of chocolate conching, *Food Funct.* 5 (2014) 235–242.
- [12] M. Kindlein, M. Greiner, E. Elts, H. Briesen, Interactions between phospholipid head groups and a sucrose crystal surface at the cocoa butter interface, *J. Phys. D: Appl. Phys.* 48 (2015) 384002
- [13] T. Nylander, R. A. Campbell, P. Vandoolaeghe, M. Cardenas, P. Linse, A. R. Rennie, Neutron reflectometry to investigate the delivery of lipids and dna to interfaces, *Biointerphases* 3 (2) (2008) FB64–FB82.
- [14] J. Penfold, R. K. Thomas, The application of the specular reflection of neutrons to the study of surfaces and interfaces, *J. Phys. Condens. Matter* 2 (1990) 1369–1412.
- [15] R. West, D. Rousseau, The role of nonfat ingredients on confectionary fat crystallization, *Critical Reviews in Food Science and Nutrition* 58:11 (2018) 1917-1936.
- [16] L. E. Scriven, Physics and applications of dip coating and spin coating, *MRS Proceedings* 121 (1988) 717. doi:10.1557/PROC-121-717.
- [17] S. Titmuss, I. Manasi, T. Saerbeck, Using Neutron Reflectivity to Understand the Structural Basis for the Rheology of Molten Chocolate (2016). doi:10.5291/ILL-DATA.9-13-642.
- [18] S. Titmuss, I. Manasi, J. F. K. Cooper, Using Neutron Reflectivity to Understand the Structural Basis for the Rheology of Molten Chocolate (2016). doi:10.5286/ISIS.E.81736474.
- [19] S. Titmuss, I. Manasi, T. Arnold, SI 15202-1: Understanding the interfacial structural basis for the rheology of molten chocolate
- [20] R. Cubitt, T. Saerbeck, R. A. Campbell, R. Barker, P. Gutfreund, An improved algorithm for reducing reflectometry data involving divergent beams or non-flat samples, *Journal of Applied Crystallography* 48 (6) (2015) 2006–2011.
- [21] T. Saerbeck, R. Cubitt, A. Wildes, G. Manzin, K. H. Andersen, P. Gutfreund, Recent upgrades of the neutron reflectometer D17 at ILL, *Journal of Applied Crystallography* 51 (2) (2018) 249–256.
- [22] A. Nelson, Co-refinement of multiple-contrast neutron/X-ray reflectivity data using *MOTOFIT*, *Journal of Applied Crystallography* 39 (2) (2006) 273–276.

- [23] A. Hughes, RasCAL.
URL <https://sourceforge.net/projects/rscl/>
- [24] R. R. L. De Oliveira, D. A. C. Albuquerque, T. G. S. Cruz, F. Yamaji, F. Leite, Measurement of the Nanoscale Roughness by Atomic Force Microscopy: Basic Principles and Applications, INTECH Open Access Publisher, 2012, London, 2012, pp. 147–174.
- [25] J. Daillant, A. Gibaud, X-ray and Neutron Reflectivity: Principles and Applications, Springer, 2009.
- [26] G. Sauerbrey, Use of quartz crystals for weighing thin layers and for weighing, GZ Physics 155 (1959) 206–222.
- [27] W. H. King, Piezoelectric sorption detector, Analytical Chemistry 36 (9) (1964) 1735–1739.
- [28] P. C. Sukanek, Dependence of film thickness on speed in spin coating 138 (6) (1991) 1712–1719.
- [29] D. Sands, Introduction to crystallography, Dover classics of science and mathematics, Dover, New York, 1993.
- [30] C. A. Beevers, T. R. R. McDonald, J. H. Robertson, F. Stern, The crystal structure of sucrose, Acta Crystallographica 5 (5) (1952) 689–690.
- [31] C. Campana Cue, A. R. Ruiz Salvador, S. Aguilera Morales, F. L. Falcon Rodriguez, P. Perez Gonzalez, Raffinose-sucrose crystal interaction modelling, Journal of Crystal Growth 231 (1) (2001) 280–289.
- [32] S. Ogawa, I. Takahashi, Chapter 6, Carbohydrate (2017) 115-130, eds. M. Caliska, I. H. Kavkli, G. C. Oz, INTECH Open Access Publisher, London.
- [33] I. Manasi, Layering of Triglycerides at Planar Sucrose Interface (2019).